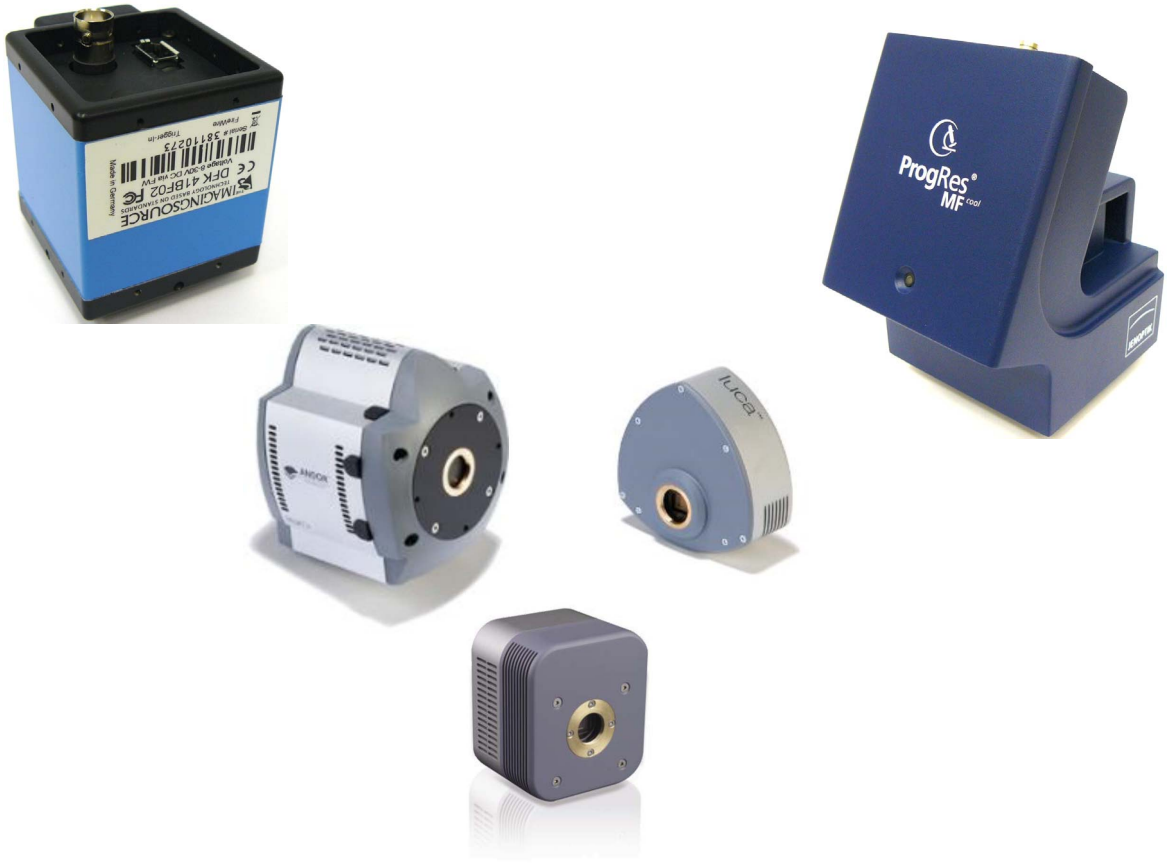
JPK Software Integration

for Cameras

User Manual

Version 2.0

06 / 2012



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§ 1 Introduction Words

This manual is mainly dedicated to introduce the camera integration into JPK Instruments systems. There are three camera systems that are fully integrated in the Software of JPK NanoWizard®, CellHesion200™ and NanoTracker™ systems. It should be explained, how the Andor cameras, ProgRess® CCD cameras, ImagingSource® CCD cameras, and µEye® USB cameras can be connected to the JPK Instrument systems and how they can be operated via the JPK software. The main camera manual should be always the provider instructions of the camera itself.

Most NanoWizard® AFM systems are equipped with a Firewire camera. The same camera can be used with either an inverted optical microscope (Life Science system) or the top view optics (standard system). If using an inverted optical microscope with the optional DirectOverlay feature, the optical image can be imported into SPM (see Main Manual Section **Fehler! Verweisquelle konnte nicht gefunden werden.**) Simply connect the Firewire cable, or USB cable from the AFM computer to the camera. Consider “video” labelled Firewire/USB ports. The connection will automatically be recognized by the software.
Start the camera viewer with the CCD button from within the JPK SPM software icon bar. The software for the optical camera is called JUnicam.

The software should automatically recognize the camera type and settings (this may take 1-2 seconds). The camera window will automatically display the live camera image.

Generally no further settings are required – the camera electronics will make an automatic adjustment of gains and exposure time that will display a reasonable image in most circumstances.

Note that the default size of the camera window is smaller in pixels than the camera chip size. This is for convenience, so it does not cover too much of the screen. The window can be increased up to the size of the chip, thereafter the image panel will not increase in size. The saved images always have the full chip size, regardless of the display size.

Please connect the USB-camera to the “video” labelled USB-port of your JPK-PC. In any other case it could be that the performance of the camera will be influenced.

§ 2 Andor Camera

2.1 Introduction

The CCD cameras from Andor Technology can be integrated into the JPK software.
The following requirements should be met:

1. A license for the Linux version of the Andor *Software Development Kit* (SDK) is a prerequisite, which is part of the software integration package that can be purchased through JPK Instruments.
2. If applicable to the specific Andor camera, the corresponding Andor *PCI interface board* needs to be transferred to the JPK PC. Obviously this requires a free PC slot, yet this should have been verified with JPK Instruments before the Andor software module was purchased. If doing this yourselves on an existing JPK PC, a few seals on the controller need to be breached. The next section deals with this hardware installation.
3. Andor camera support requires JUnicam instead of Unicam as the camera application. For Unicam users, adapting to JUnicam there should hardly be any noticeable differences. A JUnicam manual is included in this document.

Note: It is important to realize that some functions of the Andor camera may not (yet) be fully supported in the JPK software interface. In case of doubt, please contact JPK Instruments at support@jpk.com.

2.2 Hardware Installation

The main guide for hardware installation should always be the installation guide of the camera itself. This section focus on installation of Andor equipment into JPK PCs.

2.2.1 Connecting Andor Camera to JPK PC through USB

When the Andor camera to be interfaced with the JPK software is USB interfaced (such as the Andor Luca® or the Andor Clara® series), simply connect the USB cable to an available USB port on the JPK PC; preferably on the back side of the PC. No further JPK-specific hardware installation is required and the rest of this chapter can be skipped — please refer to the Andor hardware manual for further information.

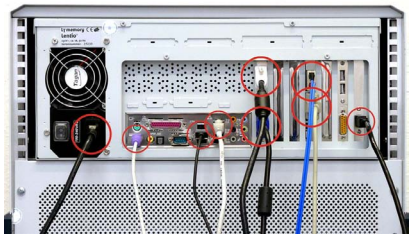
2.2.2 Installing Andor PCI Board into JPK PC within NanoWizard® Control Station

When starting to use an Andor camera with a PCI interface board in the JPK software, the PCI board first needs to be transferred to the JPK PC. **This section describes this procedure for standard JPK Control Stations.**

Open the PC
To open the PC, first shut down the PC entirely (including the toggle switch on the power supply on the rear side and disconnect all cables (see left image below). Protect the light guide cable as described in the hardware section of your main JPK product manual. Release the four screws that attach the PC to the 19-inch rack (see middle image below). Now pull the PC out of the rack.

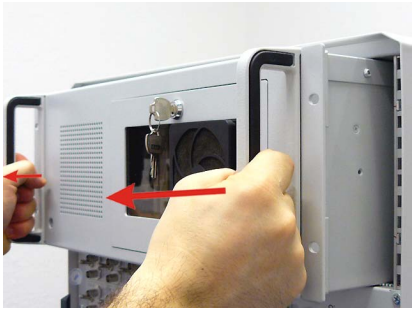
Important:

- You will have to breach a few safety seals in taking out and opening the PC.
- Mind that the PC weighs about 15 kg — do not slide the PC out solely by its handles. Instead, support the PC in the middle as well.



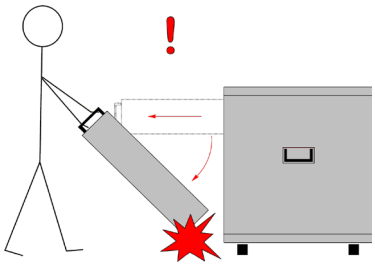
1. Disconnect all cables.

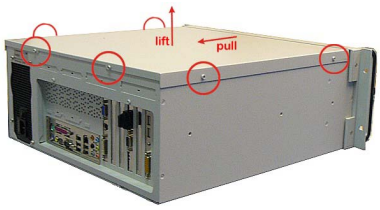
2. Release screws on front side.



3. Slide PC out of Control Station.

To open the PC cover, unscrew its 6 screws and slide the cover backwards while lifting it. Details of the PC cover may depend on the exact PC model.



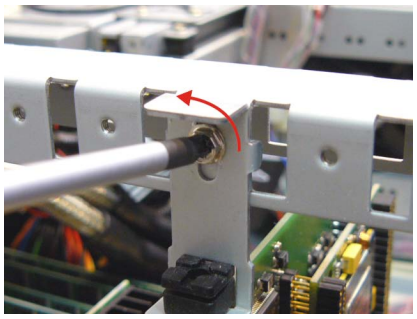


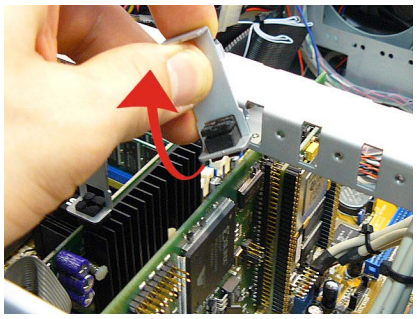
4. Open PC cover.

Install the Andor PCI board

Locate a free PCI slot on the motherboard in the section indicated in the image on the left.
Since the standard Andor PCI boards are quite large, other boards may have to be shifted for the Andor board to fit appropriately.

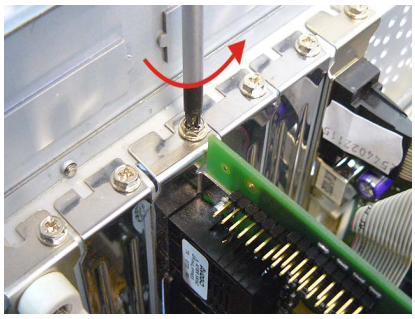
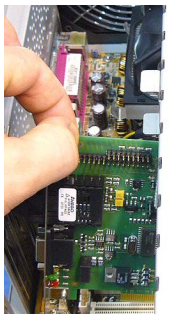
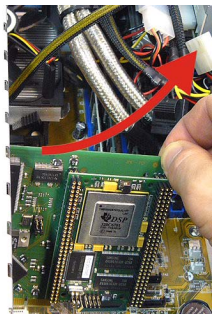
If so agreed with JPK Instruments, you may have to remove an existing PCI board at this point (such as an RS232 serial port board) to make space.



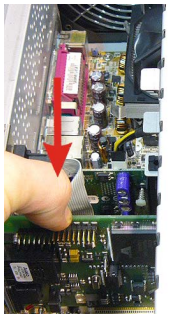
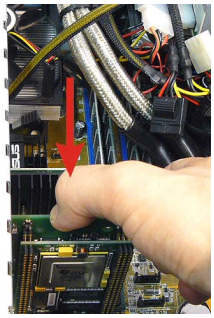


Only when moving the existing JPK DSP board:

Release the securing bracket and take it off.

Release the clamping screw on the DSP board and carefully remove it from its PCI slot from under the traverse.

Carefully put the DSP board in a new PCI slot and fix it to the PC housing using its screw. Secure the board by attaching the bracket to the traverse.

As soon as a free PCI slot is available, the Andor PCI board can be installed. To the left, the Andor CCI-23 (the default interface board for the iXon camera range) is shown.

Note: Please follow the instructions exactly as explained in the Andor manual! In particular: if so advised, make sure to attach an additional power cable from the PC power supply to the Andor PCI board.

Close the PC

Close the JPK PC and reinstall it into the Control Station by following the steps under ‘Open the PC’ above in the reverse order.

Connect the Andor camera to the PC

To connect the Andor camera to the PC, again please follow the instructions found in your Andor hardware manual.

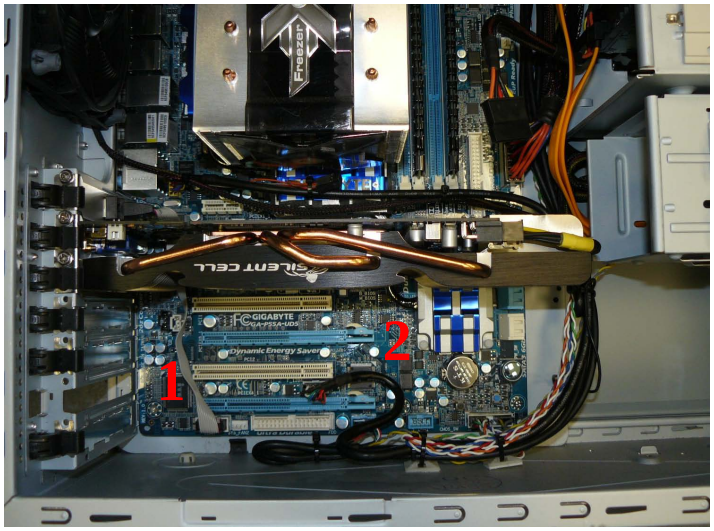
2.2.3 Installing Andor PCI Board into JPK PC for Vortis™ SPMControl Station

The procedure is similar to chapter 2.2.2. The main difference is that the PC is a stand alone system.

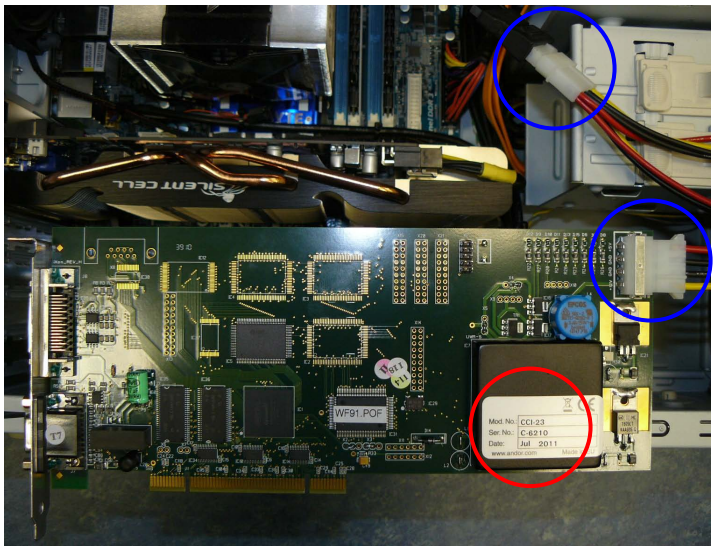
Open the PC

To open the PC, first shut down the PC entirely (including the toggle switch on the power supply on the rear side) and disconnect at least the power cable.

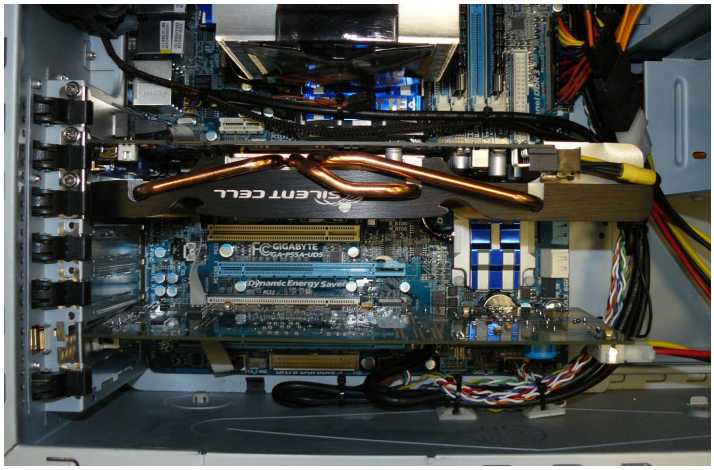
Install the Andor PCI board



Locate a free PCI slot on the motherboard (**1**- for PCI = CCl-23, **2** – PCIe *(express)* = CCl-24) in the section indicated in the image on the left.
Since the standard Andor PCI boards are quite large, other boards may have to be shifted for the Andor board to fit appropriately.



Blue circles – connect the fly lead, and splitter lead on the right place
Red circle – information about model number PCI board (CCL-23) or PCIe = XX



Carefully put the board in a PCI slot and fix it to the PC housing using its screw. Secure the board by attaching the bracket to the traverse.

Close the PC and connect all cables. Follows the instruction from Andor to connect the camera.

The e.g. iXon detector cable has to be connected to the camera as well as to the PCI board.

Note: Do not use the camera without the fan power supply.

2.2.4 Water cooling system from Koolance

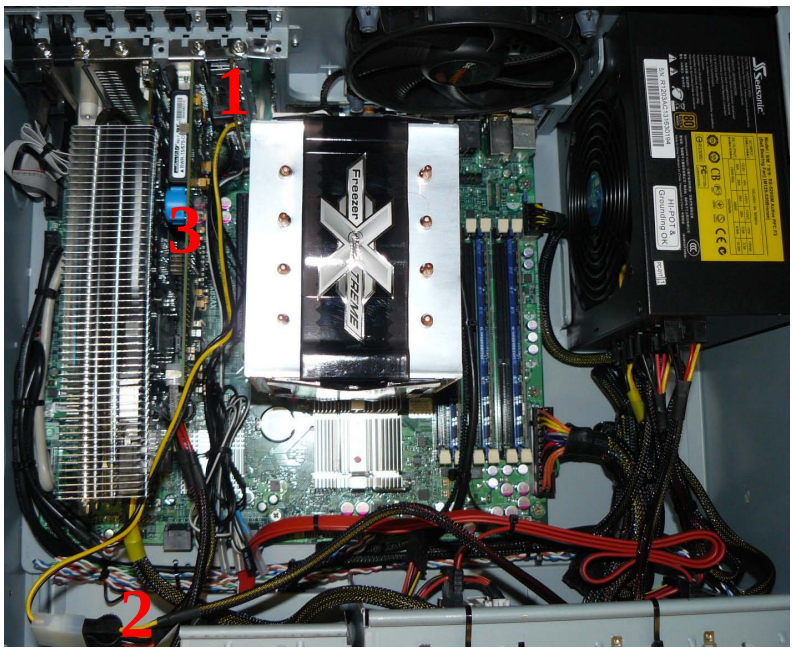
Some of the Andor cameras can be equipped with a water cooling system. This chapter describes the basic steps to install and use the water cooling system from Koolance Inc.

Please read the installation instruction for the water cooling system carefully.
Consider and follow the advices and information of Andor for use of water cooling systems.

Main parts are:

- Koolance Exos cooling system
- 2 tubing adapters (Festo; plug-screw connector, QS-1/4-6, for OD tubing 6mm)
- Tubing 2x5 m (Festo; polyurethane, PUN-H-6X1-BL, OD 6mm, ID 4mm)

Installation steps:

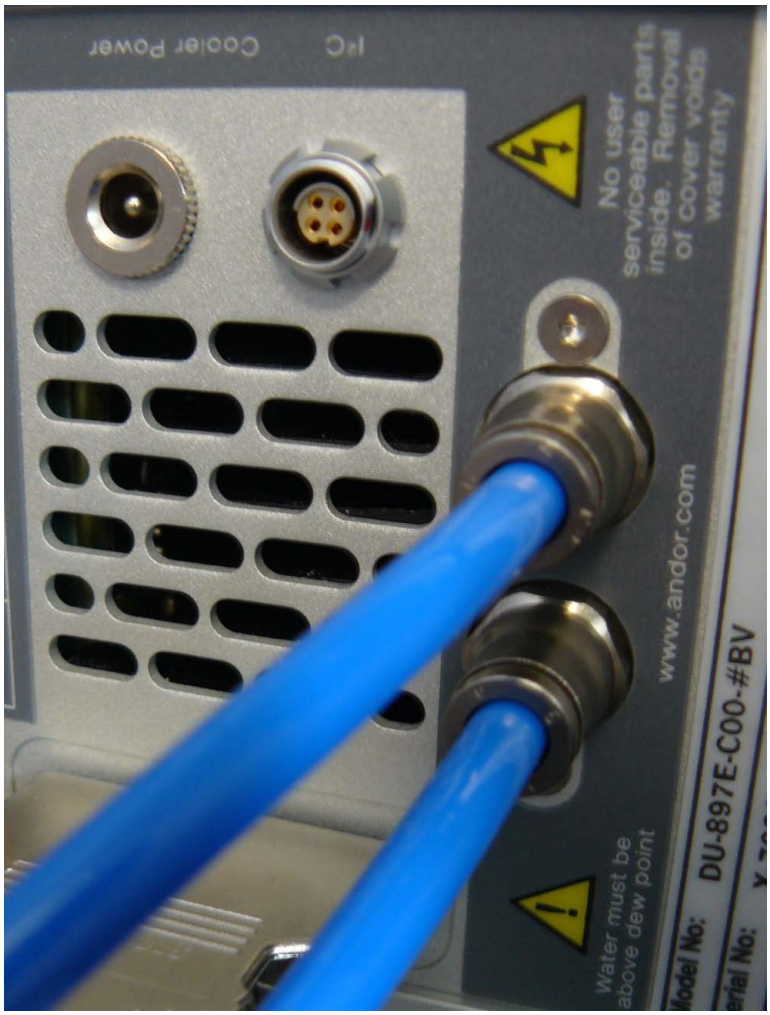


Switch off the JPK PC, disconnect the power supply.

Install the Koolance slot adapter [1] according to the Koolance user's manual. The cooling system requires 12V DC and approximately 30W. Therefore, the internal power supply can be use [2].
The PCI express board for the Andor camera [3] is visible left from the slot adapter.

Mount the FESTO tubing adapters into the threaded sockets of the cooling box. For improved sealing use PTFE tape (0.1mm thickness).

Insert the tubing into the FESTO tubing adapters. JPK support the delivery with 2x5 m tubing.



Plug as far as it will go both tubings into the related nozzles of the Andor camera.

After assembly of the tubing and the cooling system please fill the Koolance systems with liquid.

2.3 Software Installation

The JPK Andor software integration module should be purchased through JPK Instruments. All required software components will then **automatically** be included in the next software release, which can be downloaded from the JPK customers website (http://customers.jpk.com).

Note: it is not necessary to install separately the Andor SDK. In some combinations this can induce some software conflicts with JUnicam.

In particular, three Andor-specific packages are included in the installer:

andordrvlx-dkms_###.deb	Andor Camera driver for Linux
libandor2_###.deb	Packaged Andor Software Development Kit (SDK)
ucandor_###.deb	Unicap driver layer for Andor SDK

Here, ### represents the version number.

In case problems occur during the installation, please contact JPK by email at service@jpk.com or by phone at +49 30 5331 12070. Please be prepared to provide any error messages that may have occurred.

§ 3 ProgRes® CCD Camera

3.1 Introduction

The ProgRes® CCD cameras from Jenoptik can be integrated into the JPK software.

1. JPK supports MF/CF cameras as well as MF^{coo}l/CF^{cool} Firewire cameras. Additional requirements are not to be considered.
2. Jenoptik provides image capture software “ProgResCapturePro” which can be installed on a WindowsPC or MAC.

Note: It is important to realize that some functions of the ProgRes® camera may not (yet) be fully supported in the JPK software interface. In case of doubt, please contact JPK Instruments at support@jpk.com.

3.2 Software Installation

The JPK ProgRes® software integration module should be purchased through JPK Instruments. All required software components will then **automatically** be included in the next software release, which can be downloaded from the JPK customers website (http://customers.jpk.com).
Note, that it is not necessary to install separately the ProgRes® SDK. In some combinations this can induce some software conflicts with JUnicam.

Make sure that your camera is with the right firmware version – actually JPK supports the Jenoptik firmware 4.2 (13.07.2011).
If the firmware is too old or too new it can be that some features will not be supported.

In case problems occur during the installation, please contact JPK by email at service@jpk.com or by phone at +49 30 5331 12070. Please be prepared to provide any error messages that may have occurred.

§ 4 ImagingSource® Camera

4.1 Introduction

The JPK ImagingSource® software integration module should be purchased through JPK Instruments. All required software components will then **automatically** be included in the next software release, which can be downloaded from the JPK customers website (http://customers.jpk.com).

Note: it is not necessary to install separately the ImagingSource® SDK. In some combinations this can induce some software conflicts with JUnicam.

The Trigger function is available for e.g. FireWire cameras of the DFK 31BF03, DMK 31BF03, DFK 41BF02 family.

§ 5 µEye® USB Camera

5.1 Introduction

The JPK µEye® software integration module should be purchased through JPK Instruments. All required software components will then **automatically** be included in the next software release, which can be downloaded from the JPK customers website (http://customers.jpk.com).

Actually, only for some cameras of the µEye family the compatibility to JPK software was tested

- UI 1240 LE-M-GL (CMOS, 1280 x 1024, 6.784 x 5.427 mm, 25fps, Pixelsize 5.3µm,1/1.8"
- UI 1645 LE (CMOS, 1280 x 1024, 4.608 x 3.686 mm, 25fps, Pixelsize 3.6 µm, 1/3"
- UI-LE1488-M (CMOS, 2560 x 1920 QSXGA/5 MP, 5.632 x 4.224 mm, 6fps, Pixelsize 2.2µm, 1/2"

The Trigger function is available for e.g. UI-1240 ME-C or similar cameras.

If you want to use any other model of the camera family please contact JPK Instruments.

Note: it is not necessary to install separately the µEye® SDK. In some combinations this can induce some software conflicts with JUnicam.

§ 6 Software Overview: JUnicam Image Viewer

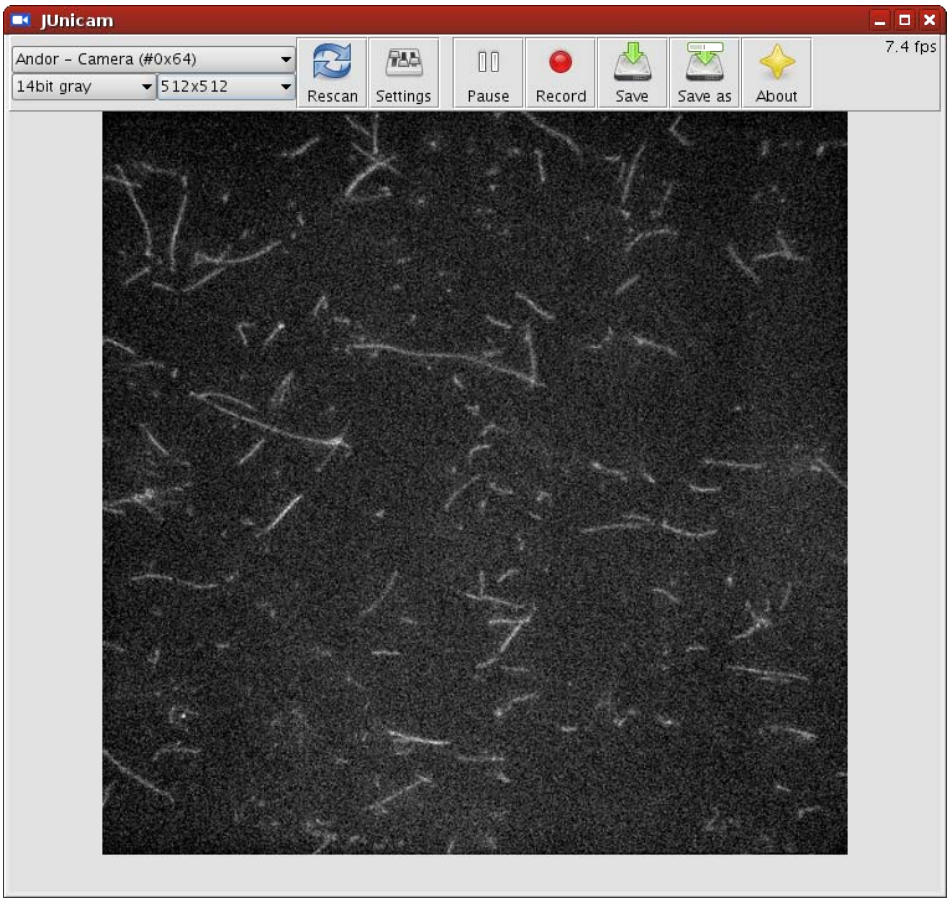
6.1 Introduction

An important component of the JPK Control Software is the live optical image viewer, called **JUnicam**. JUnicam allows live display of the current microscope-mounted camera image at video rate for a range of cameras including Andor, as well as useful interactions with the JPK software.

JUnicam can be started from the shortcut icon bar, or by selecting **CCD Camera** from the **Camera** menu.

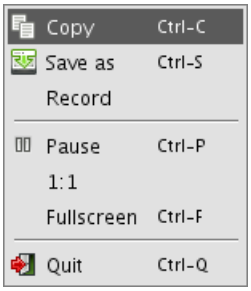
6.2 JUnicam Main Window

When started, JUnicam automatically searches for supported cameras attached to the PC. In all screenshots and corresponding descriptions below, an Andor iXon+ 897E camera (model DU-897E-C00-#BV) was used. Other actively supported cameras include several from JenOptik, Imaging Development Systems (IDS), and The Imaging Source. Please contact JPK for further enquiries under support@jpk.com or call +49 30 5331 12070.



The currently used camera is displayed on the top left. If more than one camera was found, the corresponding drop down menu can be used to select another camera. Just like in other JPK software, JUnicam has a shortcut toolbar which gives access to its most frequently used functions. In the right top corner, the current frame rate (in frames per second or fps) is shown.

If problems occur with the displaying of the camera image, the first troubleshooting step should be to press the **Rescan** button, which restarts the search for connected cameras.



Right clicking with the mouse in the image will open the menu shown on the right. A few functions are accessible through this menu. Using **Copy** will copy the current frame the clipboard, to be pasted into another program.

The **1:1** item will scale the JUnicam window to render every camera pixel on a single monitor pixel. When expanding the PC's desktop over two monitors, **Fullscreen** mode will expand the window over a single monitor.

6.3 JUnicam Software Version

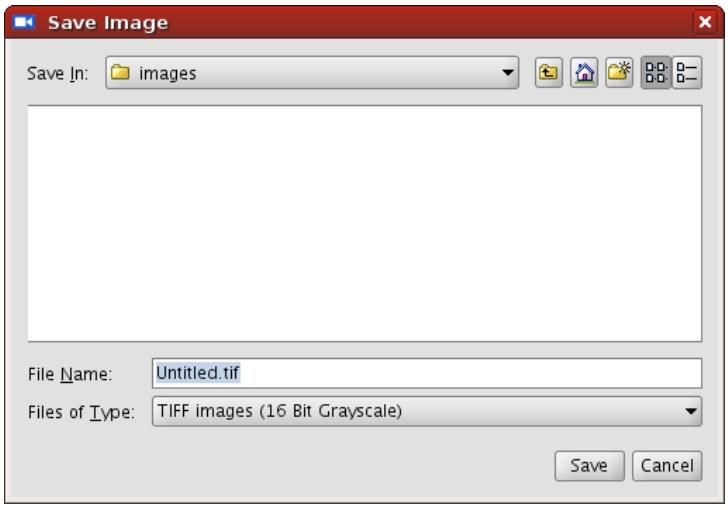
About JUnicam
JUnicam Image Capture 1.0
Display and save images and videos from video capture devices
Uses Unicap version 0.9.7
And Ucil version 0.9.7
Copyright (C) 2008 – 2010 JPK Instruments AG
http://www.jpk.com
Close

As an independent Java application, JUnicam has its own software version numbering. The current version can be found by clicking on the **About** button. This version number will be useful when contacting JPK Instruments for support.

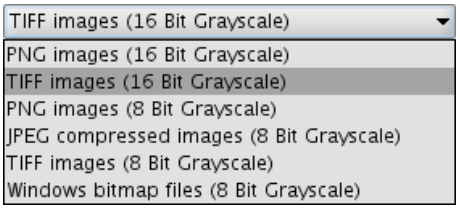
JUnicam implements a graphical user interface for *Unicap* camera drivers, the installed version number of which is also displayed.

6.4 Saving Images

Still images can be stored from JUnicam by using the **Save** and **Save as** buttons in the toolbar. The difference between the two is that the **Save** button allows for saving images with automatically incrementing filenames. The **Save as** button will always open a dialog box.



If no image has been saved during the current JUnicam session, both **Save** buttons will open the dialog shown. Images can be stored in JPEG, PNG, TIFF or BMP formats, selected from the drop-down menu.


TIFF images (16 Bit Grayscale)
PNG images (16 Bit Grayscale)
TIFF images (16 Bit Grayscale)
PNG images (8 Bit Grayscale)
JPEG compressed images (8 Bit Grayscale)
TIFF images (8 Bit Grayscale)
Windows bitmap files (8 Bit Grayscale)

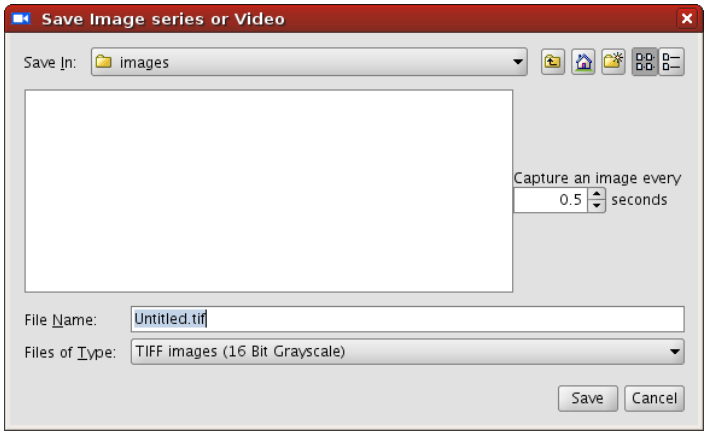
In the shown case, consecutive usage of the **Save** button will create filenames *image.tif*, *image1.tif*, *image2.tif*, etc.

Using the save buttons, images are stored exactly as displayed on-screen, including contrast enhancements.

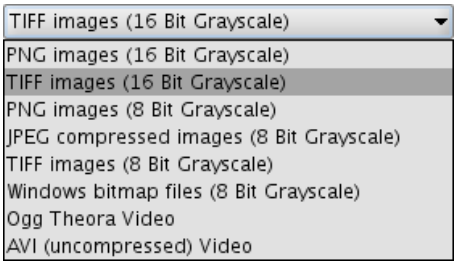
Note: Only the TIFF and PNG file formats allow storing data from cameras with a higher bit-depth than 8-bits such as from the typical 14-bit or 16-bit channels of an Andor camera.

6.5 Recording Image Sequences or Videos

Apart from still images, JUnicam can also be used to store image sequences with a fixed time interval, or even stream videos to disk into a single video file.

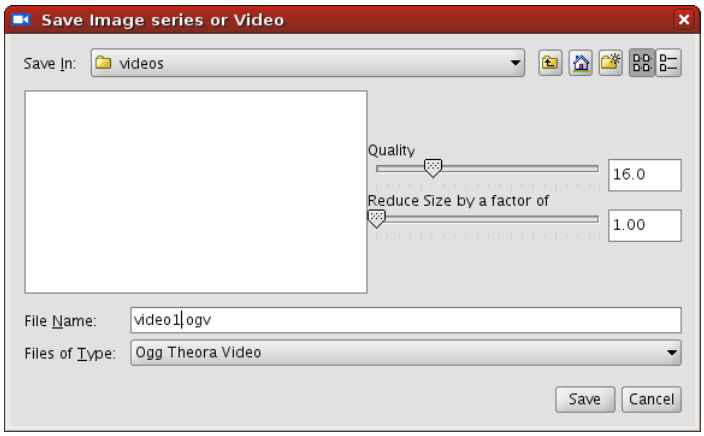


The desired video format is selected using the **Files of Type** drop down menu.


TIFF images (16 Bit Grayscale)
PNG images (16 Bit Grayscale)
TIFF images (16 Bit Grayscale)
PNG images (8 Bit Grayscale)
JPEG compressed images (8 Bit Grayscale)
TIFF images (8 Bit Grayscale)
Windows bitmap files (8 Bit Grayscale)
Ogg Theora Video
AVI (uncompressed) Video

Selecting any of the still image formats will start the recording of an image sequence. The rate at which images should be captured can be selected.

With the software version 4.0.2xx JPK supports also selected image acquisition. This mode is very useful in case of TTL-trigger supported video acquisition (**see chapter § 8**).



If the *Ogg Theora Video* format is selected, videos can be streamed to disk at the same rate at which they are being captured (up to 15 fps using most Firewire cameras).

The **Quality** setting allows control over the compression of the Ogg video file; setting it to the maximum value will store an almost lossless video.

The video size can be reduced by binning pixels using the **Reduce Size by a factor of** control. Note: This feature is currently experimental.

AVI (uncompressed) video files allow video recording without losses due to compression. Note that thus stored video files rapidly become extraordinarily large.

During the recording of a video, the **Record** button will appear as activated, and details about the recording are displayed in the bottom strip of the JUnicam window. To stop the recording, press the **Record** button again.

Please NOTE: Recording a video will only save the video with default settings of the JUnicam software. Any kind of video correction, e.g. contrast enhancement done with the JUnicam software will NOT be saved.

6.6 Splitting Videos into Single Images

Performing long term measurements it might be easier to record a video instead of single frames. The following paragraphs provide a brief description of how to split this video into single frames.

6.6.1 Using Ubuntu:

Once the data is recorded as an .avi-file using the JPK software, it is possible to have a look at the video with MPlayer or VLC Media Player. Simply open any of those programs and load the file. Make sure MPlayer is installed, because this will be the program which is used to split the video into single frames. To do so proceed as follows:

- Open a terminal window
- Go into the folder where your video is stored
- Type into the command line: 'mplayer <filename.avi> -vo png'

The video is automatically split into single frames and stored within the same folder like the video. Other output formats which are possible are .jpeg or .gif, but since the .png format offers lossless compression it is therefore recommended.

The 'mplayer video output' (-vo) command provides a lot more settings. For additional adjustments like e.g. the output directory or advanced video processing please see the manual of the 'mplayer' command.

6.6.2 Using Windows:

Once the data is recorded as an .avi-file using the JPK software, it is possible to have a look at the video with MPlayer or VLC Media Player. Simply open any of those programs and load the file. If it is necessary to do the video processing on a Windows machine, first copy the video to a Windows PC. To make sure the data is not corrupted play the video once more using VLC Media Player for Windows. The next step is to install VirtualDub (http://www.virtualdub.org) a program that will do the separation of the single frames. Simply download the file and unpack it into a new folder. The software can now be started by double-clicking on the file 'Veedub64.exe'. When all the software necessary is installed proceed as follows to split the video into single frames:

- Start the software as described above
- Open the video with 'File -> Open video file …'
- Now save the video in single frames with 'File -> Export -> Image Sequence'
- Choose a file name, a directory and an output format and press OK

The video is automatically split into single frames and stored within the chosen folder. Other output formats which are possible are .jpeg or .bmp, but since the .png format offers lossless compression it is therefore recommended.

6.7 Other Software

A few software packages not maintained or provided by JPK are available that may be useful when analyzing image or video data.

6.7.1 ImageJ

It may be convenient to use the GNU-licensed software application **ImageJ** (http://rsbweb.nih.gov/ij/) for processing of images and videos. ImageJ is also available for Ubuntu Linux and can be installed on the JPK PC. A recent version that was tested successfully by JPK is release 1.43l, which can be downloaded as a so-called Debian package from the web. Please contact JPK for further assistance.

6.7.2 Mplayer / Mencoder

Recorded Ogg videos (*.ogv) can be converted to other video formats (*.avi, *.mpg, *.wmv, etc.) using many freeware video conversion tools that can be downloaded from the internet. A good choice is the utility **mencoder**, which may be installed on the JPK PC by logging in as jpkroot, opening a terminal window and entering:

```
apt-get install mencoder
```

To convert a file INPUT.ogv to the widely used AVI format (filename OUTPUT.avi) using the *DivX* codec, type:

```
mencoder -ovc lavc -lavcopts vcodec=mpeg4 INPUT.ogv -o OUTPUT.avi
```

See the mencoder manual for more information.

§ 7 Camera Settings

7.1 Introduction

This chapter contains the description for the Andor cameras. For the ProgRes® cameras from Jenoptik as well as for the ImagingSource cameras some specifications can be very different or can be not available. In case of any doubt please read the information of the corresponding camera user manual.

When imaging using a CCD camera in low light level conditions, the key parameters of interest for the user (frame rate, image brightness, signal-to-noise ratio) are determined by a number of settings:

1. **Fixed camera properties:**
    a. sensor size / number of pixels
    b. quantum yield (efficiency of photon to electron conversion)
    c. digitization rate of analog to digital converter(s) (ADCs)
    d. dynamic range / digitization bit depth
    e. inherent noise sources (depends on most of the above properties)

2. **Adjustable camera settings:**
    a. sensor cooling
    b. exposure time
    c. camera gain (*cf.* electron-multiplication gain (EM gain) such as built into many Andor cameras)
    d. pixel readout speed (when multiple ADCs available)
    e. camera readout mode (e.g., frame transfer)
    f. total number of pixels (can be reduced from full chip by pixel binning or region of interest selection)

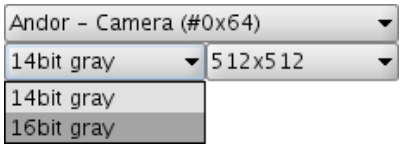
Obviously, of additional importance is the optimization of the light source that is to be imaged. In the case of low light-level **fluorescence imaging**, a delicate balance has to be found between:

1. fluorescence excitation power (related to photobleaching)
2. frame rate
3. signal-to-noise ratio in the image

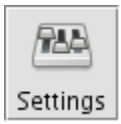
It is important to familiarize oneself with the above terms when using high-end cameras such as those from Andor Technology. In particular, some understanding of noise sources in (EM) CCD imaging is important. More information

on these and similar issues related to sensitive CCD camera usage can be found online in many tutorials and other web resources (e.g., http://www.emccd.com), or in the documentation of the Andor camera.

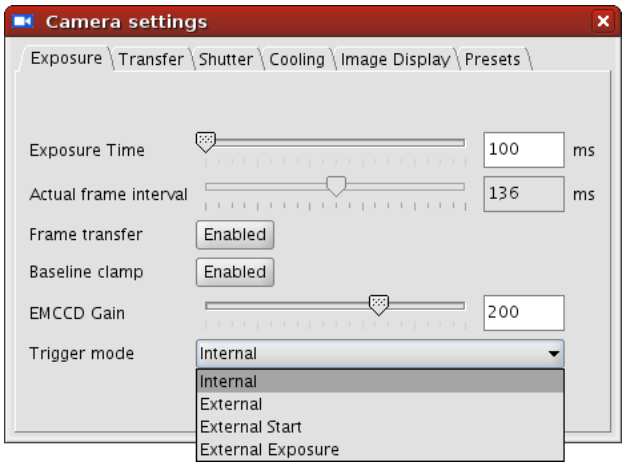
7.2 Adjusting Camera Settings



A few settings for the Andor camera are available as drop-down menus directly underneath the camera selection box: the **bit depth** and **pixel binning** settings. Note that changes in the bit depth may automatically affect other settings such as the selected ADC speed.



All further camera settings can be adjusted by opening the separate **Settings** panel from the toolbar.

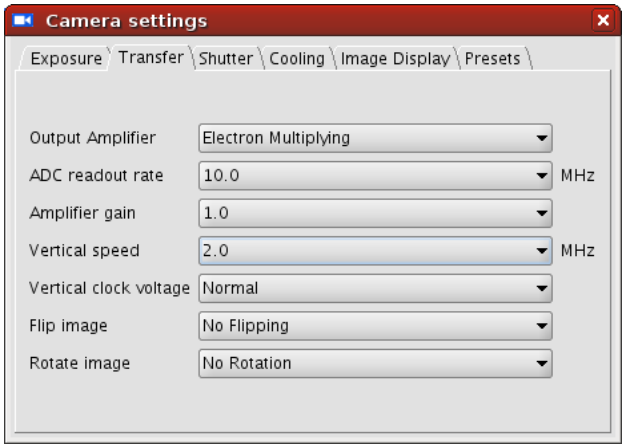


The settings panel opens in the **Exposure** tab. The **Exposure Time** is the main determinant for the frame rate. In addition, the image brightness scales linearly with this parameter. Depending on some other settings, the actual frame rate is additionally influenced by the pixel readout scheme used. This actual rate is displayed as well.

The **Frame transfer** toggle button controls whether the frame transfer feature is active. When active, acquisition of a new image can start immediately after the last one, as frame readout takes place 'in the background'. Note that in this case the **Exposure Time** should exceed the readout time – the value is automatically corrected.

The option **Baseline clamp** is only relevant when acquiring long kinetic series – please see the corresponding section in the Andor manual. The **EMCCD Gain** is set in the **Exposure** tab, too. Note that its value only affects the image when the corresponding ADC channel is selected in the **Transfer** tab (see below).

The **Trigger mode** can be used to trigger the camera with either its internal trigger signal (i.e. timing determined by software settings) or any of the external trigger modes available for the selected camera. The image shows the options for an iXon camera; see the Andor manual for further details.



The **Transfer** tab contains all settings related to the digitization of the image.

For most Andor cameras, the **Output Amplifier** can be switched between the conventional readout channel and the Electron Multiplying channel, to which the EM Gain setting from the **Exposure** tab applies.

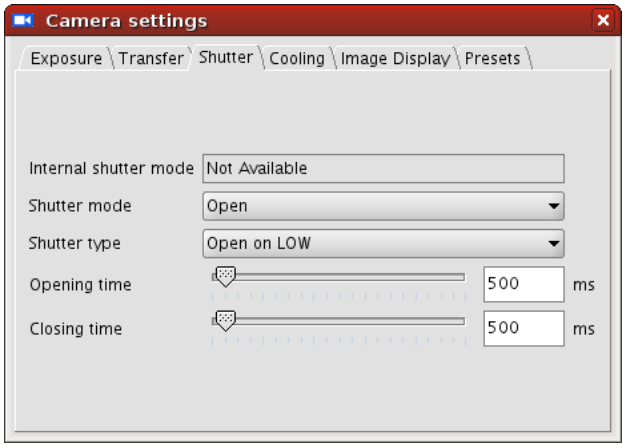
Depending on the camera model, the ADC **readout rate** can be selected from a few options. In the case of the model used for the screenshots in this manual, the Andor iXon 897E, the EM channel is equipped with 10, 5, 3 (all 14 bit) and 1 MHz (16 bit) ADCs.

Although a faster readout rate allows a higher frame rate (as the readout time per frame is shorter), one should realize that faster ADCs generate more readout noise. It is important to keep these dependencies in mind when setting up an

experiment. Again, more detailed information is found in the Andor manual.

The **Amplifier gain** can in principle always be set to the maximum value. Both **Vertical speed** and **Vertical clock voltage** will not be needed for normal operation. For more information on these properties, please see corresponding sections in the Andor manual.

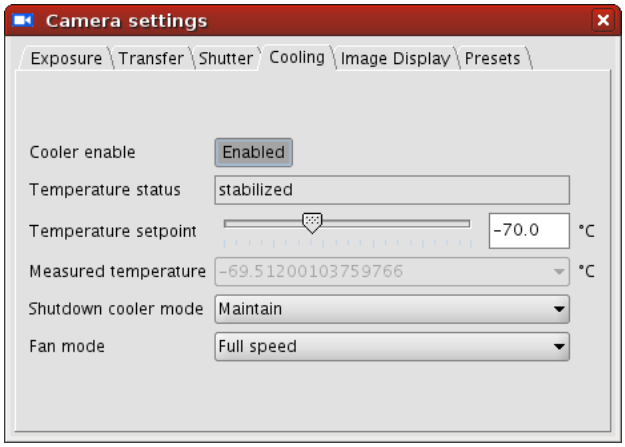
A few geometrical operations can be performed to the images read in: **flipping** or **90° rotations**. One will in principle always want to work in the orientation that fits with the real-world microscope orientation. The horizontal flipping option is most useful when switching between conventional and EM gain channels (when available), as all pixels in a row are digitized in opposite order for these channels. This property, related to the mechanical layout of the camera, gives rise to an unwanted horizontal flip.



The **Shutter** tab is mainly relevant when using a camera which has an internal mechanical shutter.

In additional, when using an external shutter, the parameters in this tab can be used to choose the right trigger settings.

See the Andor manual for more information on this feature.



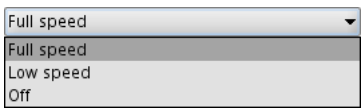
In the **Cooling** tab, the cooling settings for the Andor camera can be entered. Cooling of the camera sensor may significantly enhance the signal-to-noise ratio, which is why most Andor cameras have this built-in.

When enabled, the **Temperature status** indicates whether the control loop is 'stabilized' or not – when indicating 'not reached' or 'not stabilized', one should wait until this is the case for best results.

The **Temperature setpoint** indicates the temperature the sensor should be cooled at. Please refer to the camera specifications for details.

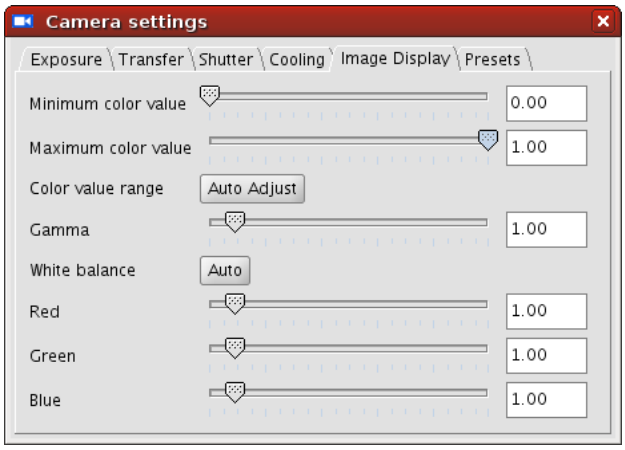
The **Measured temperature** is indicated *only when no acquisition is running*. This means that one has to temporarily pause the current acquisition using the **Pause** button in the JUnicam toolbar to visualize the actual temperature.

The **Shutdown cooler mode** determines whether the camera cooling stays on when the software is shut down.



Using the **Fan mode** drop-down menu the user can select the state of the fan that cools the camera electronics (not the sensor itself!). If the microscopy application requires this for stability reasons, one can temporarily switch the fan off.

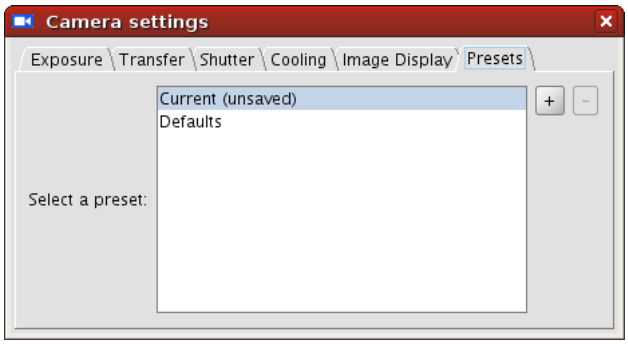
If switched off for too long, the electronics may become too hot and the camera may produce a sound alarm. See the Andor manuals for more information on this. Switching off the fan for extended periods of time is only allowed when using a camera cooled using a water recirculator.

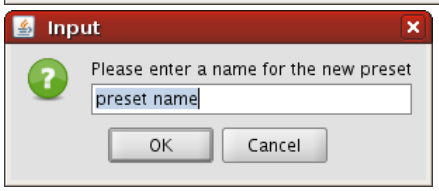


The **Image Display** tab contains further parameters that only change the display and storage behavior of the image rather than physical camera parameters. The display contrast can be tuned by changing the **Minimum** and **Maximum color value**s. The **Auto Adjust** button for the color value range is useful to quickly find the right contrast settings.

These contrast values directly affect the values in image files (video files are NOT affected) that are stored by JUnicam.

(Note that the difference between the settings on this tab and those in the other tabs is that here only display-specific; no device-specific settings are shown.)



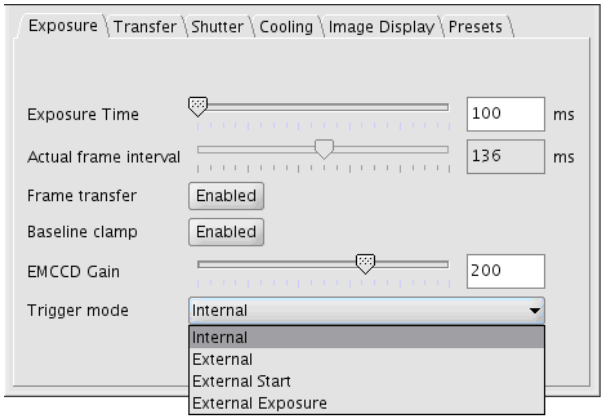


Camera settings may be saved and loaded on the **Presets** tab. The parameters of all tabs will be stored. After clicking on the **+** button, a dialog will pop up which allows to type a name to describe the current settings (e.g., 'fluorescence' or 'all manual'). This text will then be displayed in the presets list, which allows to rapidly and reproducibly switch between sets of values.

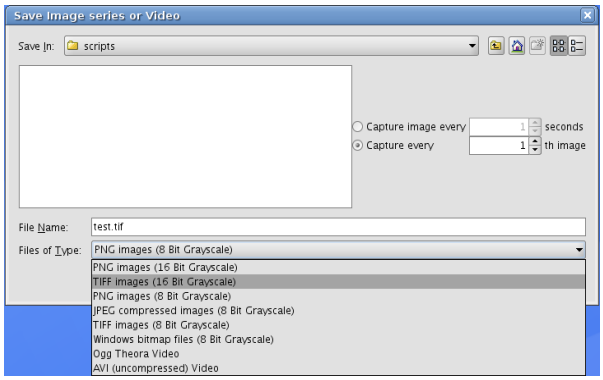
The stored presets are retained when the program is closed.

7.3 Camera Settings - Andor

To run TTL-trigger supported image acquisition 1) the Andor camera has to be connected via **TTL-trigger input**, 2) the Trigger mode has to be set to **External**, and 3) the video capture has to be set to "**Capture every *x.*. th image**".



Change the trigger mode from Internal to External.

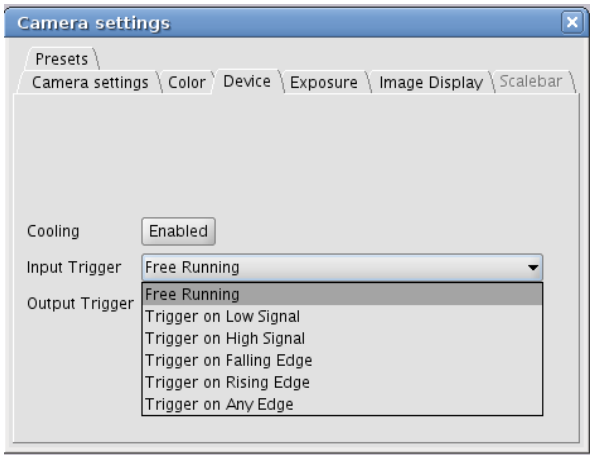


"**Capture image every xxx seconds**" – in that configuration the video sequence starts with the first TTL- trigger. Therefore, only one trigger signal is necessary for each sequence. To stop the image sequence click on the "**Record**" button

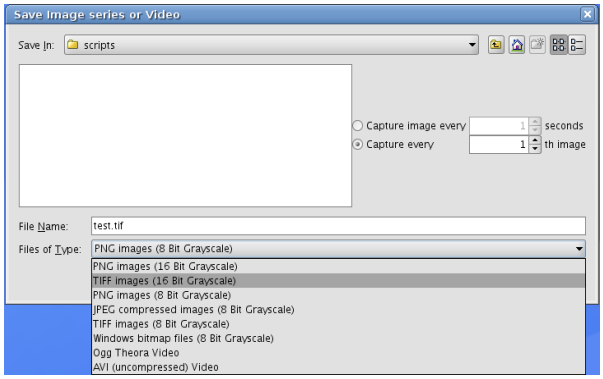
"**Capture every xxx image**" – in that mode every TTL-pulse will induce an image acquisition. Click the "**Record**" button again to stop the sequence.

7.4 Camera Settings - ProgRes® Camera

TTL-trigger supported image acquisition is quite similar to Andor: 1) the ProgRes® camera has to be connected via **TTL-trigger input**, 2) the Trigger mode has to be set to e.g. **Trigger on Rising Edge**, and 3) the video capture has to be set to "**Capture every *x.*. th image**".

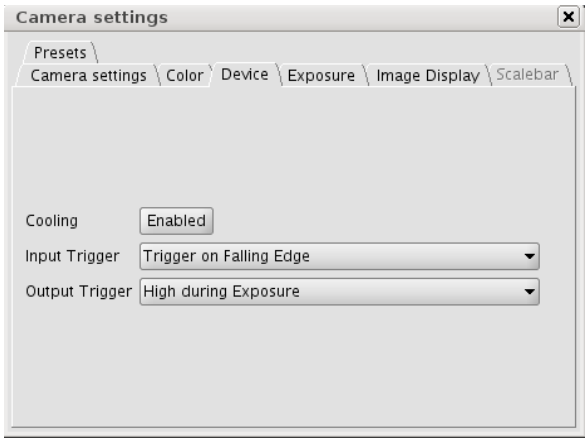


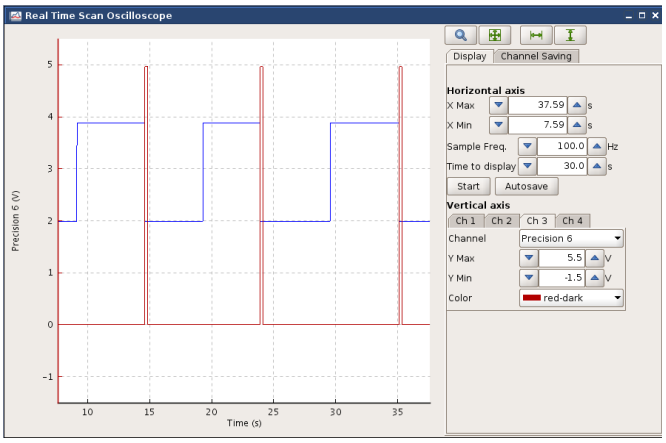
Change the trigger mode from Free Running to Trigger on Rising/Falling/Any Edge.



"**Capture every xxx image**" – in that mode every TTL-pulse will induce an image acquisition. Click the "**Record**" button again to stop the sequence.

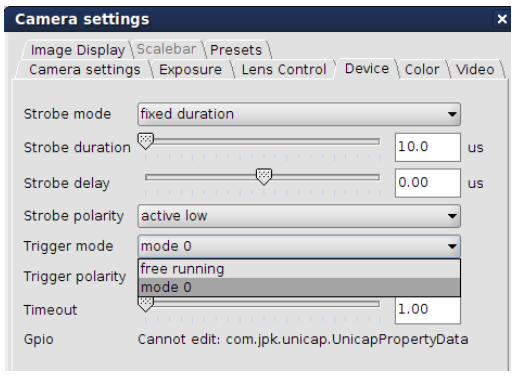
Some ProgRes® cameras allow to send a Trigger out signal (see pin connection in 8.1). The camera settings has to be changed for Trigger Output to e.g. on "high during Exposure". To display the output trigger the Precision 6 channel of ANALOG IN can be used. The example monitors trigger in signal (in blue) and corresponding trigger output signals (in red).



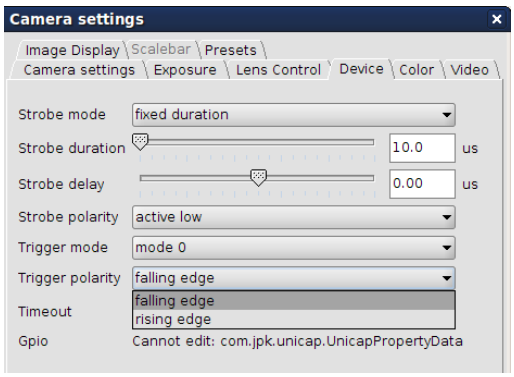


7.5 Camera Settings - ImagingSource®

TTL-trigger supported image acquisition is quite similar to Andor and ProgRes®: 1) the Trigger mode is set from "free running" to "mode 0", 2) the Trigger polarity has to be set to e.g. **Falling Edge** or **Rising Edge**, and 3) the video capture has to be set to "**Capture every *x.*. th image**".



Change the trigger mode from **free running** to **mode 0**; **Trigger polarity**: **Falling** or **Rising Edge**.



"**Capture every xxx image**" – in that mode every TTL-pulse will induce an image acquisition. Click the "**Record**" button again to stop the sequence.

§ 8 TTL Trigger for Image Acquisition

TTL (transistor-transistor logic) devices are wildly used in lots of electronic applications and can be used to synchronize the NanoWizard AFM with external hardware components like cameras. A particular characteristic of TTL signals is that they can switch between a low state (below 1 V for a digital 0) and a high state (above typically 3.3 V for a digital 1) in a high switching speed. This is called level change. A combination of a fast switch between low and high state leads to a pulse segment.

8.1 Hardware Connection

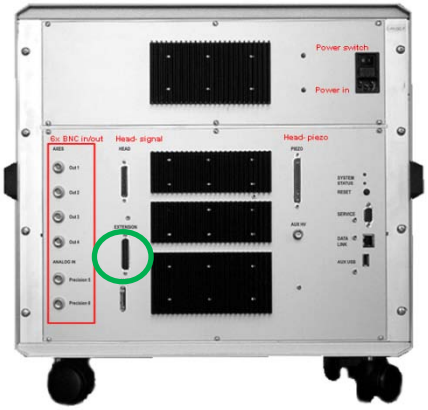
The Vortis controller unit for the JPK NanoWizard III can be equipped with and without a signal access module (SAM). The full range of TTL control elements can only be used with the signal access module.

NanoWizard® controller without SAM

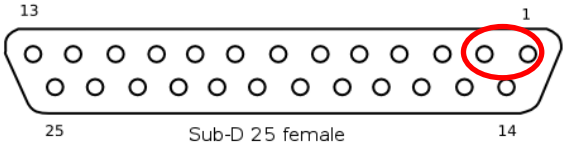
NanoWizard® controller with SAM

TTL Signal without SAM

The access to the TTL signal is given at the back of the controller via a Sub-D 25 female pin assignment (marked in green). The standard Vortis controller without signal access module offers the possibly to use two TTL outputs (marked in red).



Extension mixed signal Sub-D 25 female pin assignment:



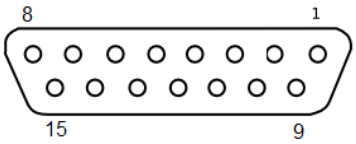
Pin	Assignment
1-2	TTL (level change)

Note: The TTL outputs (Pin 1/2) can switch between high and low state (level changer), but there is no possibility to generate automatic pulses.

TTL Signal with SAM

The access to the TTL signal is given at the signal access module of the controller via a Sub-D 15 female pin assignment (marked in green). Next to the switches between high and low state, automatic pulses can be generated. Furthermore TTL pulses can be synchronized with AFM measurements through pixel, line and frame clocks.

Extension mixed signal Sub-D 15 female pin assignment:



Pin	Assignment
1-2	ground (no TLL output)
3	TTL (level change, pulse)
4	TTL (level change, pulse, frame clock)
5	TTL (level change, pulse, line clock)
6	TTL (level change, pulse, pixel clock)
7-9	ground (no TLL output)
9-11	TTL (level change)
12	ground (no TLL output)
13-15	TTL (level change)

8.1.1 Andor Camera

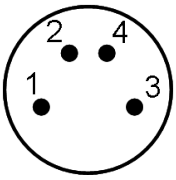
Use the SMB to BNC cable from Andor.

Connect the SMB-cable to the external trigger port of the camera.

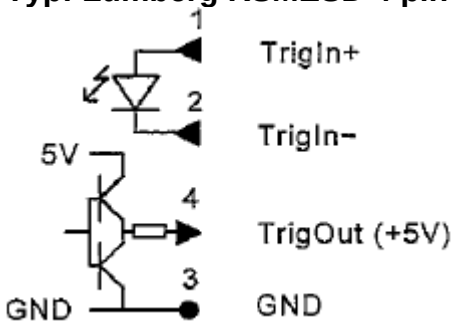
8.1.2 ProgRes® Camera

Please follow the instructions of the ProgRes® user manual. To connect the Trigger port of the Jenoptik camera the plug connector RKMCK4 (www.lumberg.de) is needed.

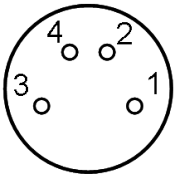
Trigger/Flash
Camera male plug



Typ: Lumberg RSMESD 4 pin



Female plug socket



8.1.3 ImagingSource® Camera

The ImagingSource® cameras can be simply triggered with a standard BNC-cable.

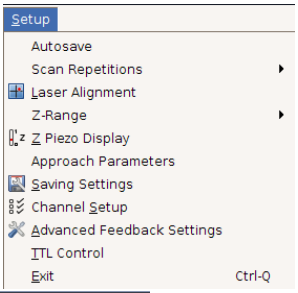
8.2 Software Configuration

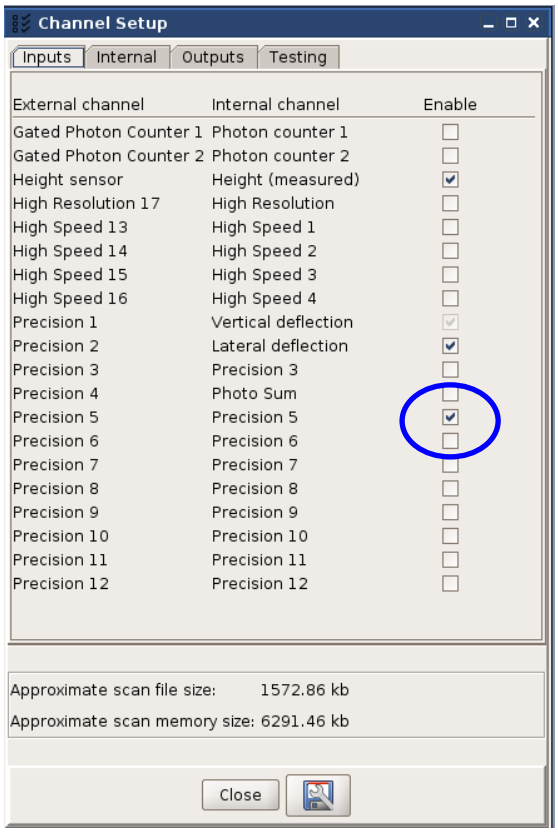
To monitor the TTL signals with JPK software tools it is necessary to activate:

1. Channel Setup/Inputs/Precision 5 (if the signal input is Precision 5)
2. Open the Real Time Oscilloscope/Channel saving – activate Precision 5

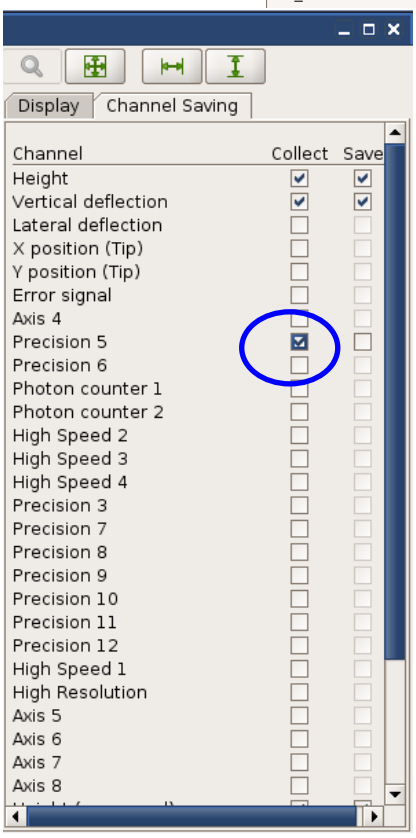
To send a TTL signal it is possible to use any of the following options:

3. SPM Jython console and type in:
   a. dspManager.setDigitalOutPin(0,1,True) to switch on the TTL pulse
   b. dspManager.setDigtalOutPin(0,1,False) to switch off the pulse for pin10 = PT1 at the TTL-output plug
4. ExperimentPlanner with an example script e.g. ”TTL-multiple.py”
5. TTL control via TTL control panel and with Force RampDesigner™ (see NanoWizard main manual sections 8.4.2. and 8.4.3, or CellHesion 200 manual section 4.11.; the TTL control panel is NOT yet finished for the NanoTracker™)





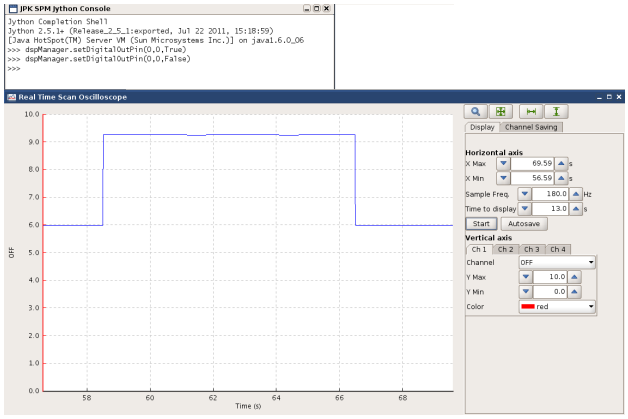
Channel Setup

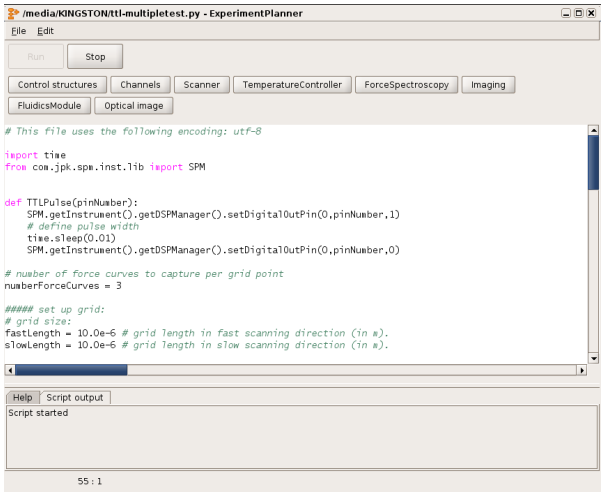


Channel saving of Real Time Oscilloscope

Jython commands to activate and de-activate the TTL pulse. In this example the pin 9 = PT0 was used for output.

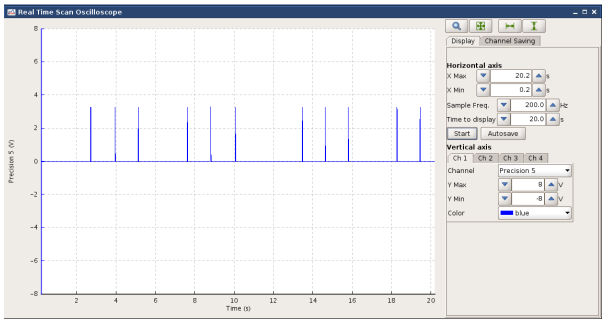
Real Time Oscilloscope to monitor the TTL-pulse.





ExperimentPlanner – this example uses a TTL trigger to force spectroscopy on a grid at the beginning of each force curve.

In that example for each grid point 3 force curves will be collected.



The Real Time Oscilloscope displays the corresponding sequence of TTL-signals for 3 force curves per grid point.

§ 9 Camera Adapters

C-mount adapters are necessary to assemble the camera on the camera-port of the microscope body. These lens couplers can have different magnifications. The right optics depends on the chip size of the used camera.
The following couplers can be used exemplarily for the different camera types:

1) ImagingSource® camera
   e.g. DMK 31BF03, DFK 31 BF03
   1/3" chip = 0.3x or 0.5x coupler

2) ProgRes® camera
   e.g. MFCool, CFCool
   2/3" chip = 0,63x or 0.7x coupler

3) Andor camera
   e.g. Ixon 897, Clara, Luca
   1" chip = 1x coupler (without internal lens)
   1/3" chip 1/3 inch chip = 0.3x or 0.5x coupler

Note: the C-mount adapter will change the magnification, e.g. using a 0.6x coupler for the ImagingSource® camera instead of the standard 0.5x coupler the object within the camera image appears slightly increased as compared to what is visible within the eyepieces.

Note: All trademarked names mentioned in this manual remain the exclusive property of their respective owners.

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